PUPAL DIAPAUSE OF *COLORADIA PANDORA* BLAKE (LEPIDOPTERA: SATURNIIDAE)

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Abstract.—Pupae of the pandora moth, *Coloradia pandora* Blake, were collected in central Oregon and stored at 5° C for 8 to 24 weeks, then incubated at 25° C. The minimum cold storage time required to break diapause was 12 weeks, but emergence rates were highest (87.5%) for 14–18 weeks. In a separate experiment, 1000 pupae were maintained in field enclosures for 3 years while soil temperature was monitored. Seventy-two percent of these pupae emerged in Year 1. Soil temperature fell below 5° C for 21.7, 22.9, and 25.1 weeks over the three consecutive winters, and the minimum soil T was −2° C. In the lab study, ≥22 weeks at 5° C limited emergence to <40%, therefore mortality from duration of cold could be considerable in winters such as Year 3. Prolonged (extended) diapause was observed in only 0.6% of the sample population.

Key Words.—Insecta, *Coloradia pandora*, pandora moth, pupae, diapause, phenology, rearing, adult emergence.

The pandora moth, *Coloradia pandora* Blake, a native defoliator of *Pinus* spp. in the western U.S., has a biennial life cycle throughout most of its range (Tuskes et al. 1996). The pupal stage generally lasts 12 to 13 months (June through July the following summer in central Oregon) (Ross 1995, 1996), although pupal diapause as long as 6 year may occur (Carolin 1971). The proportion of the population remaining in diapause beyond 1 yr has been estimated at <5% (Massey 1940) up to "a substantial part of the generation" in some areas (Carolin & Knopf 1968). A recent outbreak of *C. pandora* in central Oregon was characterized by alternating summers of severe defoliation, and summers with dense moth populations. If a substantial proportion of an epidemic population remained in extended diapause, then conspicuous defoliation and moth flights during off-years would be expected, however, this was not observed.

The life histories of abundant, episodic forest insects can influence ecological processes such as predator-prey relationships and host tree physiology. In the case of *C. pandora*, a high incidence of extended diapause would diminish the biennial nature of its life cycle, possibly increasing defoliation stress on host pine trees and enhancing the availability of *C. pandora* pupae as prey for insectivores. Therefore, one objective of this study was to quantify the occurrence of extended diapause in central Oregon. To do this, we tracked emergence of a single cohort of pupae for three years under natural conditions.

The second objective of this study was to determine the length of cold storage required for successful pupation of *C. pandora*. We were interested in minimizing pupal diapause for the purpose of rearing adult moths in the laboratory. Massey (1940) attempted to break diapause by exposing pupae to low temperatures in December, then incubating the pupae at 21–27° C. He did not specify the cold storage temperature or duration of exposure, and his pupae required 4 months

exposure to high temperatures before any development was noted. Time to emergence generally decreases with the duration of exposure to cold, provided the temperatures are within an acceptable range for the species (Danks 1987). Our cold storage experiment, in conjunction with the extended diapause study, provided some insight about environmental conditions that could promote extended diapause in *C. pandora*, and conditions that could cause mortality in the pupal stage.

METHODS AND MATERIALS

Cold Storage.—Pupae were collected 1 November 1992 in an area of the Deschutes National Forest, Oregon, that had been heavily defoliated the previous spring based on USDA Forest Service Region 6 aerial surveys. The pupae were covered with sandy soil and refrigerated at 2 to 5° C for a minimum of eight weeks. After eight weeks, eight female and eight male pupae [sexed according to characteristics of the fourth and fifth abdominal segments (Tuskes et al. 1996)] were transferred to 4 liter paper buckets with screen tops, covered minimally with sand and incubated at 25° C in a rearing room with a 16:8 light:dark photoperiod. Another 8 female and 8 male pupae were removed from cold storage at 12, 14, 16, 18, 20, 22 and 24 weeks. Soil in the refrigerator and sand in the rearing room were misted with water several times weekly. Female and male emergence was monitored daily.

Extended Diapause.—Pupae were collected on 15 June 1995 from 2 locations in Pringle Falls Experimental Forest, about 45 km SW of Bend, Oregon. These sites had been moderately to heavily defoliated by the current generation, but not the previous generation of *C. pandora* based on USDA Forest Service Region 6 aerial surveys, so the pupae were unlikely to be more than 1 year old. Two hundred pupae were laid in the bottom of each of 5 screen enclosures (0.9 × 0.9 × 0.9 m) and covered with ≈6 cm top soil. Three of the enclosures were located within the Pringle Falls Research Natural Area in a mature stand of ponderosa pine, and the other 2 enclosures were placed 4 km distant at the Pringle Falls Research Forest Headquarters under a similar canopy. Adult emergence was tallied by gender for each enclosure on 3 or 4 dates in July and August from 1995 through 1997 (Year 1–Year 3). A soil and air temperature monitor (Omnidata Datapod® model DP-212, Logan, Utah) was installed in November 1994, to document exposure to cold temperatures in the first winter of pupal diapause prior to collecting the pupae, and for the remainder of the experiment. The soil temperature probe was buried at the same depth as the pupae (6 cm), and the air temperature probe was placed next to one of the enclosures at the Headquarters location. The Datapod® recorded maximum, minimum, and average daily temperatures.

RESULTS AND DISCUSSION

Cold Storage.—No adults emerged from the cohort receiving 8-weeks cold storage, even after 177 days at 25° C. Successful completion of diapause was first observed for pupae in the 12-week treatment group (Fig. 1). Optimal survival rates (87.5%) were achieved with 14 to 16 weeks at 5° C for males and 16 to 18 weeks for females. Female emergence lagged behind that of males, indicating protandry (Fig. 1). The length of time to emergence at 25° C declined with in-
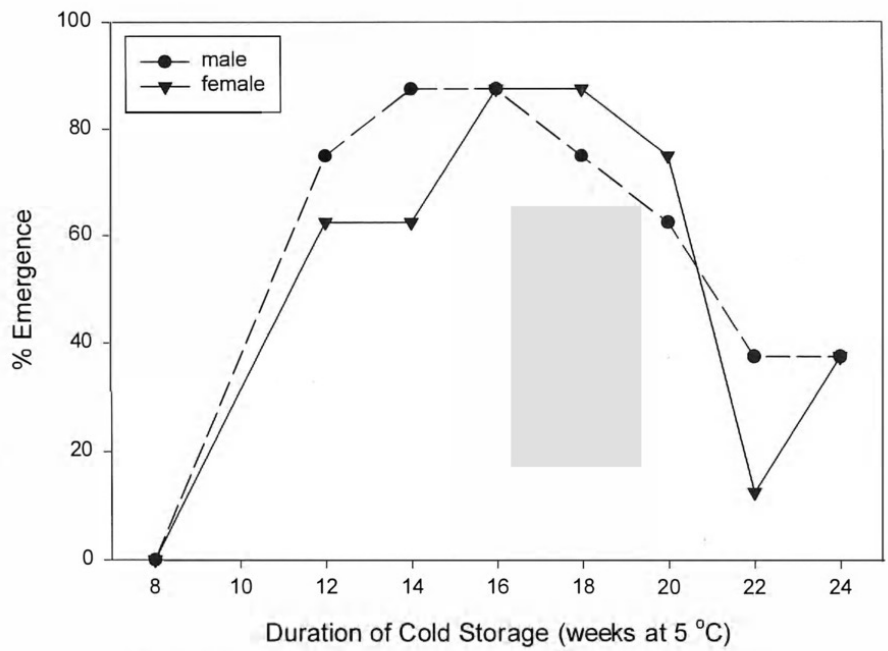
Figure 1. Percent *C. pandora* adult emergence relative to length of cold storage at 5° C. *n* = 8 female and 8 male pupae per time period.

creasing time in cold storage (Fig. 2). Pupae stored for 24 weeks at 5° C required 1 month less time at 25° C to complete diapause compared to pupae in cold storage for 12 weeks. Linear regression of time to emergence on the length of cold storage revealed that, for each additional week of cold storage, the incubation time declined by 3 days. Thus in a laboratory situation, additional cold storage beyond the minimum required to break diapause would not be time efficient, except that the rate of successful emergence was not constant (Fig. 1). The uni-
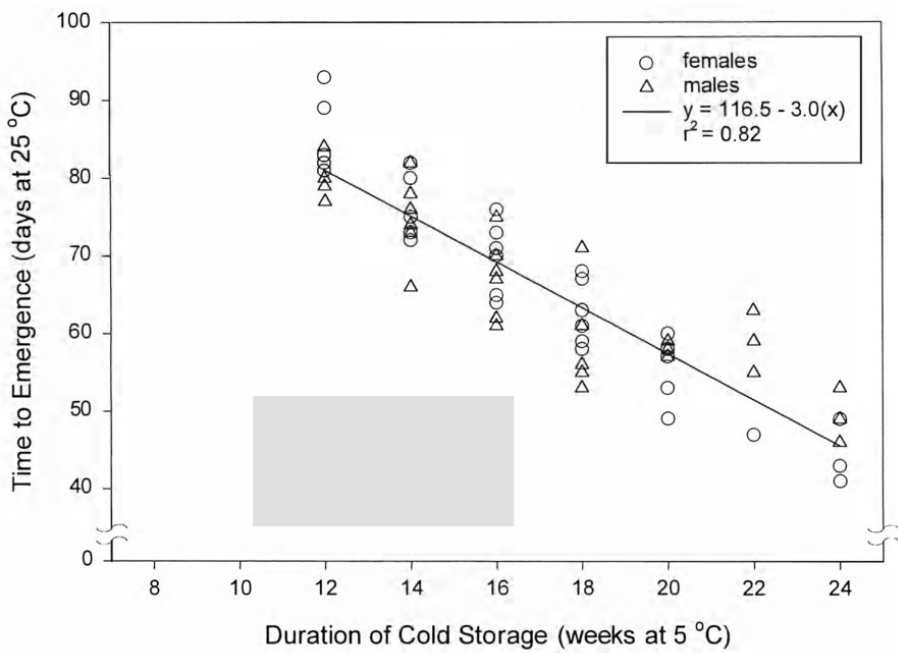

Figure 2. Incubation time at 25° C needed for *C. pandora* adult emergence relative to duration of 5° C cold storage period of pupae.

Table 1. Emergence rates of *Coloradia pandora* adults for 5 enclosures, each containing 200 pupae.

Enclosure	Year 1 %	Year 2 %	Year 3 %	Total %
A	74.0	0.5	0	74.5
B	70.5	0.5	0	71.0
C	73.5	0.5	0	74.0
D	75.0	0	0	75.0
E	66.5	1.5	0	68.0
Mean	71.9	0.6	0	72.5

form incubation temperature of 25° C was selected to favor rapid development, rather than to mimic field conditions. Soil temperatures measured in the field during the extended diapause experiment never exceeded 23° C.

There is a cold duration threshold between 8 and 12 week below which *C. pandora* pupae from this population are physiologically unable to complete diapause. Half of the pupae in the 8-week cold treatment group were dissected following the experiment and were found to be undifferentiated and apparently still viable. Because this experiment did not incorporate cold storage treatments between 8 and 12 week, we cannot determine the threshold more precisely. Twelve weeks should be considered minimal because the pupae in this experiment may have had some prior exposure to temperatures $<5°$ C. They were collected from the field on 1 November 1992, and subsequent monitoring in 1995 and 1996 for the extended diapause experiment indicated that soil temperatures may drop below 5° C in the later half of October. Therefore, the actual length of cold treatment these pupae received could have been several weeks longer than treatments imposed in the lab.

Cold exposure in the pupal phase may not be prerequisite to adult emergence throughout the range of *C. pandora*. There are three geographically disjunct populations of the nominate subspecies (Tuskes et al. 1996). Central Oregon is the northern extent of the western distribution which extends south into California and Nevada. By several accounts (Aldrich 1921, Tuskes 1984), some adults in the isolated population of southern California have been observed emerging after just 2 months of pupation. Significant latitudinal variation in pupal diapause is common (Danks 1987, Tuskes et al. 1996). For example, the saturnid *Automeris io* (Fabr.) produces both diapausing and non-diapausing pupae in the lower latitudes of its range (Manley 1993).

Extended Diapause.—In Year 1, 67 to 75% of the pupae in the 5 enclosures successfully completed diapause (Table 1). An additional 0 to 2% emerged in Year 2, and none emerged the 3rd year following pupation, indicating extended diapause was rare in the sample population. Most of the pupae remaining in the enclosures after 3 years were desiccated, a few were moldy, and 4 appeared potentially viable upon dissection. Total emergence rates in the field enclosures (grand mean = 72.5%, Table 1) fell below the maximum successful pupation rates of the cold storage experiment (87.5%, Fig. 2), but this may be explained by soil temperatures. Soil temperatures averaged $\leq5°$ C for at least 152 days (21.7 weeks in Year 1 (Fig. 3A), so based on results from the cold storage experiment, we might expect successful pupation rates as low as 20–40% (Fig. 1). If the
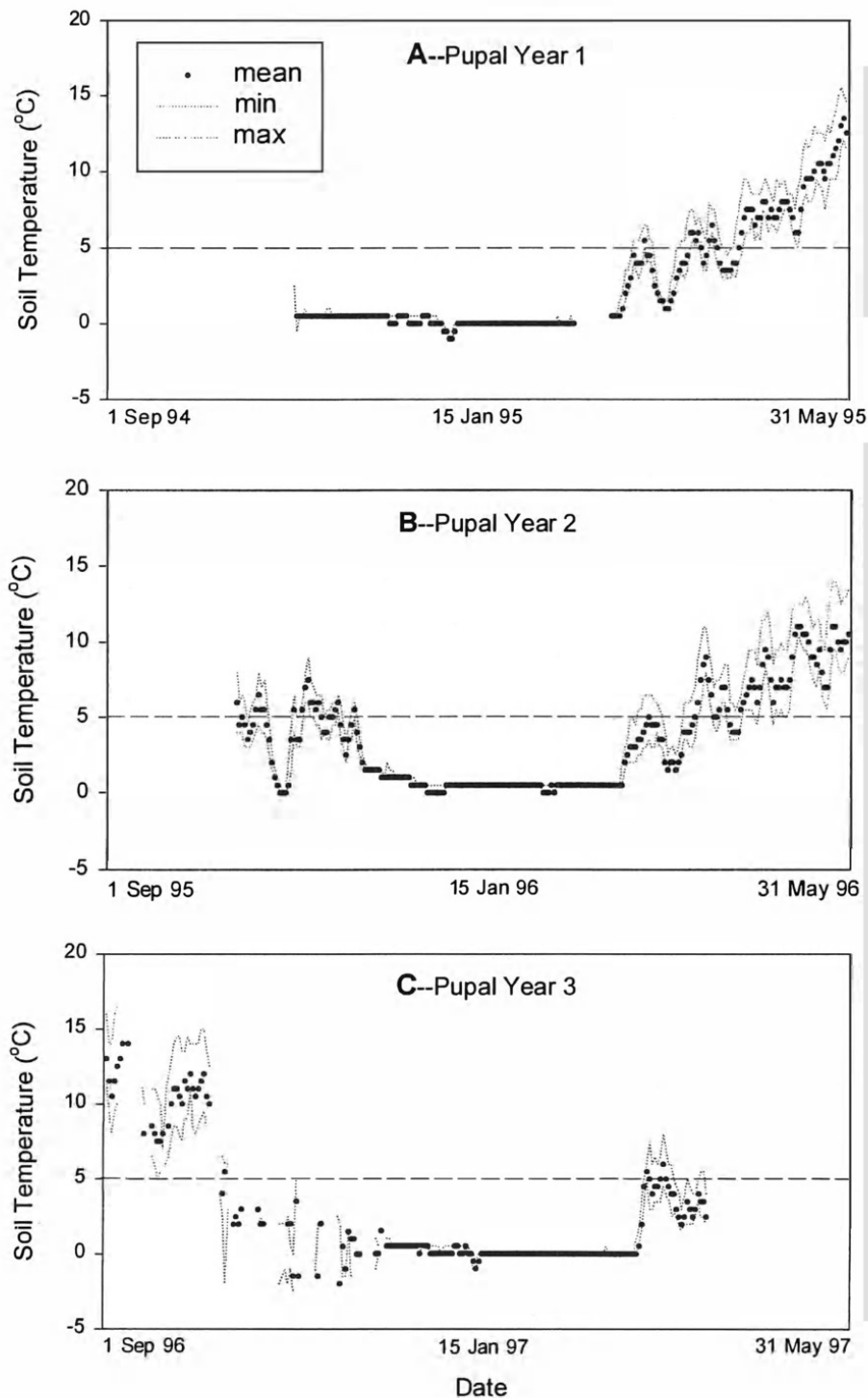
Figure 3. Daily soil temperatures at Pringle Falls Headquarters, Deschutes National Forest, Oregon. Temperature probe at 6 cm depth. (**A**) 1st year, (**B**) 2nd year, (**C**) 3rd year of pupal diapause.

pupae used for the cold storage experiment actually did have 1–2 weeks additional exposure in the field prior to collection, then the expected successful pupation rate for 22 weeks at 5° C would be 63–75%, which corresponds well with the emergence rate from the extended diapause experiment. During the winter of Year 2 there were 22.9 weeks with soil temperatures ≤5° C, and in Year 3 there were 25.1 weeks ≤5° C (Fig. 3B, 3C). These data, along with the cold storage results,

suggest that the duration of cold soil temperatures in central Oregon probably restricts the maximum successful pupation rates for *C. pandora*, perhaps as much as 60% over a long winter.

Winter mortality in other moth species is a function of degree, as well as duration, of cold (Turnock et al. 1983). Diapausing *A. io* pupae tolerate air temperatures in excess of $-18°$ C, but freezing temperatures extend the duration of diapause (Manley 1993). The degree of sensitivity of *C. pandora* to freezing temperatures is unknown. In the first year of the extended diapause experiment, the minimum recorded soil temperature was $-1°$ C and 72.5% of the pupae survived. Over 3 winters, soil temperatures hovered around $0°$ C for extended periods (Fig. 3) despite marked fluctuations in air temperatures, thus it seems likely that duration, rather than degree of cold, would cause winter mortality under typical field conditions. *Coloradia pandora* pupate about 6 cm below the soil surface in central Oregon which buffers exposure to cold. Snow cover can dramatically reduce winter mortality from freezing temperatures (Turnock et al. 1983), but persistence of the snow pack in spring could lengthen pupal exposure to cold by slowing soil warming. The insulating effects of leaf cover and snow have been shown to influence pupal development of *A. io* (Manley 1993). An open forest canopy that intercepts less snow in winter and allows for rapid snow melting in spring might be favorable to *C. pandora* pupation. Decreased canopy cover (Ross 1995), lower elevation and southerly aspect (Schmid 1984), all of which hasten melting of snow and possibly reduce the duration of cold soil, are known to promote earlier emergence of adult *C. pandora*. There is also some evidence that water from snowmelt may trigger developmental processes in diapausing insects (Danks 1987).

Synchronized emergence and protandry are characteristics of the temporal emergence patterns of lepidopteran populations that favor successful mating (Tuskes et al. 1996). *Coloradia pandora* appears to utilize both strategies to some extent. Moths emerge throughout a 30- to 40-day period, but the peak emergence period lasts about 7 days (Schmid 1984, Ross 1996). Overall emergence in our field enclosures was fairly coincident in Year 1 as 95% of the moths which emerged did so within a 2-week period (Fig. 4). More frequent observations may have narrowed this time frame. Adult *C. pandora* may live 7–10 days (Schmid 1984, personal observation) so this peak period is relatively broad compared to some shorter lived saturniids having synchronized emergence within several days (Tuskes et al. 1996). Protandry slightly offsets the synchrony of emergence toward males first, which may benefit the population by increasing the likelihood that females are able to mate and lay eggs soon after emergence (Tuskes et al. 1996). In Year 1 of the extended diapause study, 85% of male emergence and 70% of female emergence occurred by July 28 (Fig. 4). Protandry also was observed in the cold storage experiment (Fig. 1) and in other *C. pandora* studies (Massey 1940, Schmid 1984). A total of 299 females and 420 males emerged in Year 1, suggesting the sex ratio in the population may have been biased in favor of males. However, the one-thousand pupae in the enclosures initially were not sexed, so it remains possible that higher mortality rates in female pupae caused fewer adult females to emerge.

Coloradia pandora pupae are food for golden-mantled ground squirrels (*Spermophilus lateralis* Say), white-footed deer mice (*Peromyscus maniculatus* Wag-
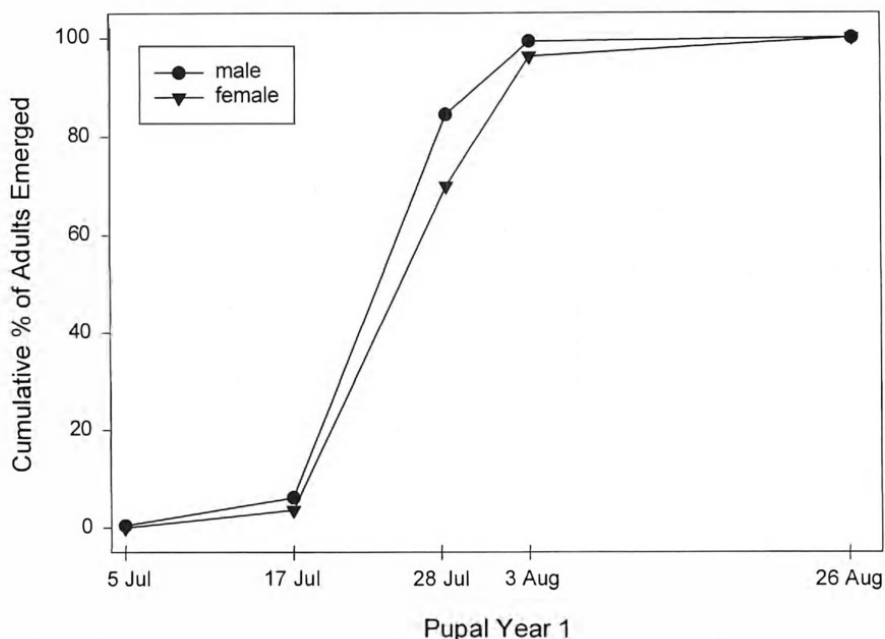
Figure 4. Cumulative emergence pattern for adult *C. pandora* moths. Percentages are based on the total number of males and females emerged in the 1st year of pupal diapause.

ner), and possibly other wildlife in central Oregon (unpublished data). Extended diapause in a biennial insect would provide a more reliable prey base for insectivores utilizing only the pupal stage. Fecundity and survivorship of wildlife with short lifespans (1–5 year), such as deer mice and ground squirrels, may be influenced by annually fluctuating food resources. In our study, less than 2% of pupae remained viable (i.e., edible) beyond 1 year of development, hence they would constitute a biennial food source in central Oregon. Carolin's (1971) paper on extended diapause has been interpreted as indicating that *C. pandora* provided an annual food base for aboriginal Paiute tribes in California, despite the biennial cycle of *C. pandora* (Weaver & Basgall 1986; but see Fowler & Walter 1985, Blake & Wagner 1987). In areas with milder winters (< 12 weeks below 5°C), *C. pandora* pupae could possibly hold over a second year in order to acquire the requisite cold period to complete diapause; but it is also possible that univoltinism created an annual supply of larvae in southern California (Tuskes et al. 1996).

Geographical differences in environmental regimes may explain Carolin's (1971) observations of extended diapause. He also collected pupae in central Oregon, but he held them over winters in Portland, Oregon which has a relatively mild, maritime climate. Carolin's data showed 18% of pupae emerged in their 2nd year, and another 37% emerged the 3rd year. However, even more important is the fact that his initial population of pupae were culled from unemerged pupae remaining after a large flight year. Therefore, the extended emergence rates Carolin reported are not applicable to whole populations.

information and convenience of the reader and does not constitute official endorsement or approval by the U.S. Department of Agriculture.

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